International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 1, Feb 2015, 1-10

ANTIMICROBIAL SCREENING OF CALLUS EXTRACTS OF *CANTHIUM PARVIFLORUM* LAM

SIRIGIRI CHANDRA KALA[1], KOKKANTI MALLIKARJUNA[2], PATCHALA ARUNA[3] & MUVVA VIJAYALAKSHMI[4]

[1]Research Scholar, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

[2]Assistant Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

[3]Lecturer in Microbiology, D. K. Government Degree College for Women, SPSR Nellore, Andhra Pradesh, India

[4]Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

ABSTRACT

A large number of medicinal plants have been extensively screened for biologically active molecules with the intension of finding new drugs for treating different diseases. To determine the antimicrobial activity potential of leaf callus cultures of *Canthium parviflorum*, the present study is designed to screen and identify the therapeutic suitability of this plant extract for the treatment of a particular disease. The callus from the leaf explants of the species were induced on Murashige and Skoog basal medium supplemented with various concentrations of 2, 4-D. The callus was extracted sequentially with hexane, chloroform, ethyl acetate and methanol for 24 h by using Soxhlet apparatus. The inhibition of zone mean values were statistically analyzed with the MINITAB 14 by the general one way (un stacked) analysis of variance (ANOVA) to find out the most effective extracts. The extracts were exposed against Gram positive and Gram negative bacteria as well as fungi by agar well diffusion method at 100 ppm concentrations. Among four extracts, chloroform and methanol extracts showed high zone of inhibition in all tested bacteria. Methanol extract generated high zone of inhibition in all tested fungi. The present study indicated that the callus extracts of *Canthium parviflorum* has potent antimicrobial compounds, which of these extracts need to be isolated and characterized. The plant leaf callus can be further subjected to enhancement and isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

KEYWORDS: *Canthium parviflorum*, Callus Extracts, *In vitro* Culture, Antimicrobial Activity

INTRODUCTION

Medicinal plants play a vital role for the development of new drugs. Plants and plant derived products are part of healthcare system since ancient human civilization. Medicinal plants are playing an important role in producing the therapeutic drugs with minimal or without side effects compared to synthetic drugs [(1)] In order to find new sources of plant drugs, number of plants has been screened for various biological activities in various research institutions. Discovery of natural substances has been showing the path to do the extensive work on medicinal plants. Since their discovery during 20th century, antimicrobial agents have substantially reduced the threats posed by infectious diseases. The use of these

"wonder drugs" has lead to a dramatic drop in deaths from diseases that were previously wide spread, untreatable and frequently fatal. These drugs have also contributed to the major gains in life expectancy. Most of the microbial agents are less susceptible to regular antibiotics and recover of increasing resistant isolates during antimicrobial therapy is on rise throughout the world which highlights the need for new antibiotics with broad range and potent activity.

Canthium parviflorum Lam. (syn: *Plectoria parviflora*) of Rubiaceae is commonly called as Balusu in Telugu, thorny subscandent shrub distributed throughout India in dry plains. *Canthium parviflorum* has wound healing properties,[(2)] traditionally used for snake bites, [(3)] and leaf paste used for scabies and the ring worm infection.[(4)] This plant contains high quantities of carotenoids and beta carotenoids which are very essential for vitamin A activity. *Canthium* as herbal medicine is used for the treatment of diabetes among major tribal groups in South Tamilnadu.[(5)] Phytochemical analysis revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, steroids, saponins, terpenoids, sanranetin-4-o-glycoside long chain acids and cardiac glycosides in *Canthium* leaf extracts. [(6)] Thorns of this plant have been found to contain taraxerol, d-mannitol, petunidin and long chain esters. *Canthium parviflorum* is the richest source of β-carotene and 100g edible portion of fruit contains carotenoids (9.51mg) and β-carotene (6.10mg).[(7)] In the wild plant, extracts of *Canthium parviflorum,* contains alkaloids, oils, flavonoids, gums, phenols, saponins, steroids, tannins and terpenoids.[(8)] *Canthium parviflorum* is an important medicinal plant used in indigenous system of medicine in India and abroad. Though the medicinal importance of this plant is known, phytochemical and pharmacological basis is not known. Hence, by keeping this in mind, callus from leaf is developed and compounds from it are extracted into various solvents, which are screened against broad spectrum of microbes.

MATERIALS AND METHODS

Plant Material

The fresh plants of *Canthium parviflorum* were collected from Acharya Nagarjuna University Campus, Guntur District. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (Teepol), Bavistin for 3 min and Mercuric Chloride (0.1% w/v) for 1 min, followed by 70% ethanol. These leaf explants cultured on Murashige and Skoogs medium (1962) [(9)] supplemented with different concentrations and combinations of phytohormones used for callus production.

Callus Culture

MS basal medium was prepared and the leaf explants were cultured with various concentrations of 2, 4-D for callus induction. Preferred concentration for callus induction is 2, 4-D 2.0 mg/l. Old callus was collected after 30-60 days and sub cultured on fresh medium with same combination of growth regulators twice in four week time interval.

Extraction from Callus Cultures

Leaf calli of 6-8 week old derived from the leaf cuttings were collected and dried in an oven at 40±1º for 5h. The dried calli was collected, homogenized to a fine powder and stored in airtight bottles. Shoot calli powder of 25 g was extracted with solvent of 150 ml of each of hexane, chloroform, ethyl acetate and methanol for 24h by using Soxhlet apparatus. All the prepared extracts were concentrated separately on rotary evaporator at 40^0 C. The extracted powder was re dissolved in the same solvent and 100mg/ml were prepared. These crude callus extracts is used for anti-microbial activity.

Tested Bacteria

Gram positive bacteria- *Bacillus megaterium*, *Bacillus subtilis* (ATCC- 6633), *Pseudomonas aeruginosa* (ATCC-9027), *Staphylococcus aureus (*MTCC 3160), *Lactobacillus acidophilus* (MTCC 447) and *Lactobacillus casei* (MTCC 1423).

Gram negative bacteria- *Escherichia coli* (ATCC-35218), *Streptococcus mutans* (MTCC- 497), *Enterococcus faecalis* (MTCC- 439), *Proteus vulgaris* (MTCC 7299), *Xanthomonas campestris* (MTCC 2286) and *Salmonella typhi* (ATCC 14028).

Tested Fungi

Fungal - *Candida albicans* (ATCC-10231), *Fusarium solani* (MTCC- 6773), *Fusarium oxysporum* (MTCC-3075), *Aspergillus niger* (MTCC-872), *Helminthosporium solani* (MTCC–296), *Rhizoctonia solani* (MTCC-4634), *Trichoderma viride* (MTCC–793) *and Botrytis cinerea* (MTCC–359).

Antimicrobial Assay

The antimicrobial activity of the crude extracts of *Canthium parviflorum* was determined by agar well diffusion method of Cappuccino and Sherman [10] and Volk *et al*, [11]. To culture the test bacteria Nutrient agar and Czapek-Dox agar media were used. NA medium of 100ml was prepared and sterilized at 15 lbs pressure ($121^{O}C$) for 15 min and cooled 0.2 ml of test bacterial suspension was inoculated on the medium. After inoculating the seed medium was thoroughly mixed and poured into petri plates under aseptic conditions. After the solidification of the agar medium, 4mm diameter wells were punched into it with sterilized cork borer. For the antifungal assay, spore suspension of test fungus (105 Spores/ ml) was mixed with the cooled, molten CD agar medium and poured into Petri dishes. After solidification wells were made in the medium by sterilized cork borer. The crude extract which was dissolved in hexane, chloroform, ethyl acetate and methanol at a concentration of 25, 50, 75 and 100 ppm was added to each well. Streptomycin served as positive control while hexane, chloroform, ethyl acetate and methanol served as negative control for both bacterial and fungal strains. Fluconazole (0.1 ppm/well) served as the positive experimental control for all fungal strains assayed.

Statistical Analysis

The mean values were statistically analyzed with the MINITAB 14 by the general one way (un stacked) analysis of variance (ANOVA) to find out the most effective extracts and the most sensitive test organisms. Differences between antimicrobial assays were scored by ANOVA followed by LSD ($P<0.05$).

RESULTS & DISCUSSIONS

Antibacterial Activity

To minimize the maximum usage of the medicinal plants, it became an urgent need to propagate the plant in less time and space. *In vitro* culture is only option left behind. In current study we attempted to propagate *Canthium parviflorum* by *in vitro* culture, carried out phytochemical, antibacterial and antifungal studies against callus and reported for the first time. The callus was extracted sequentially with hexane, chloroform, ethyl acetate and methanol. Each extract was tested for antibacterial (Table 1) and antifungal (Table 2) activities.

As observed in the case of wild *Canthium* plant, callus extracts also showed potent antibacterial and antifungal activity against a broad range of microorganisms. Among the four solvent extracts of callus, chloroform extract showed high zone of inhibition in all the tested bacteria (Table 1). The ethyl acetate callus extract produced high zone of inhibition in *B. subtilis* and *S. mutans* (Table 1). Less zone of inhibition is formed by hexane extracts in 8 species of bacteria. But *Pseudomonas aeruginosa* has shown high zone of inhibition (5.26-15.32 mm) with hexane extract.

The *Canthium* plants showed more potent of antibacterial activity on both Gram positive and Gram negative bacteria. The low and high zones of inhibitions observed in microbes are as follows, *Bacillus megaterium* (3.24–22.80 mm) (Figure 1A), *Bacillus subtilis* (3.32-20.42 mm) (Table 1) (Figure 1B), *Lactobacillus casei* (2.45-22.42 mm) (Table 1) (Figure 1D), *Pseudomonas aeruginosa* (5.26-15.32 mm) (Table 1), *Lactobacillus acidophilus* (4.56-14.56 mm) (Table 1) (Figure 1C), *Staphylococcus aureus* (5.36-20.56 mm) (Table 1) (Figure 1E), *Streptococcus mutans* (Table 1) (5.28-25.34 mm), *Enterococcus faecalis* (2.26-18.28 mm) (Table 1), *Escherichia coli* (2.60-18.84 mm) (Table 1), *Proteus vulgaris* (2.45-20.55 mm) (Table 1) (Figure 1F), *Xanthomonas campestris* (4.82-19.28 mm) (Table 1), *Salmonella typhi* (2.24-14.68 mm) at 25 -100 ppm (Table 1). As the concentration increases to 100 ppm the zone of inhibition also increased. The high zone of inhibition observed in all these bacteria could be due to more secondary metabolites being in it as observed in qualitative phytochemical analysis. In all these cases, the zone of inhibition increased with increase in the concentration of extract.

Antifungal Activity

The callus extracts of *Canthium* plant exhibited very potent activity against all the fungal species tested. Among the four solvent extracts, methanolic extract produced high zone of inhibition in all the fungal species tested (Table 2). Minimal zone of inhibition of many fungi is formed by chloroform extracts (Table 2). The low and high zone of inhibition of all fungal species is as follows, *Fusarium solani* (2.54-29.54 mm) (Table 2), *Fusarium oxysporum* (4.33-35.25 mm) (Table 2), (Figure 2A), *Rhizoctonia solani* (6.25-29.60 mm) (Table 2), (Figure 2B), *Trichoderma viride* (2.55-25.66 mm), (Table 2), (Figure 2C), *Helminthosporium solani* (2.50-30.15 mm) (Table 2), (Figure 2G), *Botrytis cinerea* 6.72-29.24 mm (Table 2), (Figure 2F), *Candida albicans* (6.35-17.55 mm) (Table 2), (Figure 3 D,E) and *Asergillus niger* (2.65-14.55 mm) (Table 2) (Figure 2H). As the concentration increases to 100 ppm the zone of inhibition also increased.

Natural products of higher plants may give a new source of antimicrobial agents possibly with novel mechanisms of action. Contrary to the synthetic drugs; antimicrobials of plant origin are not associated with many infectious diseases in different parts of the world. [12] Several researchers reported the valuable phytochemicals and antimicrobial activities from medicinal plants, present in the *in vitro* condition. Several reports on use of callus for preliminary screening of phytochemicals, antimicrobial activity and pharmacological studies from *Striga hermonthica,*[13] *Bacopa monnieri* L,[14] *Centella asiatica,*[15] *Centella asiatica,*[12] *Premna serratifolia,*[16] *Ocimum basillicum,*[17] *Biophytum sensitivum,*[1] shoot callus from *Passiflora edulis* [18] was observed.

In the present study, potent antibacterial activity against both Gram positive and negative bacteria is exhibited by *Canthium* callus extracts. Among all the extracts, chloroform and methanol extracts has produced high zone of inhibition in all the bacteria tested might be due to more number of compounds dissolved it. In hexane extract, it showed less zone of inhibition. Chloroform extract showing high zone of inhibition is reported from *Passiflora edulis.*[19] Qualitative analysis of callus extracts supports the notion that the activity is due to more number of dissolved compounds.[20] Ethyl acetate extract of *Canthium parviflorum* callus extracts showed high zone of inhibition in two species of bacteria. The *Canthium* extracts

also showed high potent activity against all tested fungal species. Among the four extracts, methanol extract generated high zone of inhibition in all the fungi. Chloroform extract also showed high antifungal activity. Such a kind of methanol and chloroform extracts showing potent activity is reported from *Alchornea* plant [21].

Previous reports on wild plant leaf material of *Canthium parviflorum* showed that it contain very low antimicrobial activity against *E. coli* and various selected bacteria and ineffective against *Enterococcus foecalis, Klebsiella pneumoneae, Klebsiella oxtoca, Proteus mirabilis, Staphyloccus aureus and Pseudomonas aeruginosa.*[22] Another report on wild plant material of *Canthium parviflorum* exhibits high antimicrobial activity against the Gram positive (*B. subtilis* and *S. aureus*), Gram negative strains (*E. coli* and *K. pneumoniae*) and antifungal activity against *A. niger, A. fumigatus* and *C. albicans*[8] was observed. *Canthium dicoccum* have good *antimicrobial activity.*[23] The ethanol extract of *Paederia foetida* L. (Rubiaceae) leaf extracts showed significant antimicrobial activity against Gram-positive (*Staphylococcus aureus amd Enterococcus faecalis*) and Gram-negative (*Escherichia coli, Salmonella typhimurium* and *Shigella flexneri*) bacteria.

Priya *et al*, in 2009 reported antimicrobial activity of leaf extract of *Canthium parviflorum* plant on *E. coli* only. The mature leaf extract of *Canthium* failed to show any activity against *E. foecalis, Staphylococcus aureus and Pseudomonas aeruginosa.*[22] Peter *et al*, in 2011[8] reported the preliminary phytochemical and antimicrobial screening of wild whole plant extract of *Canthium* and reported that ethyl acetate extract has shown potent activity on Gram+ strain (*B. subtilis* and *S. mutants*) and Gram– strain (*E. coli* and *K. pneumoniae*). He also reported antifungal activity of ethyl acetate extract on against *A. niger, A. fumigatus* and *C. albicans*. But in the present study, *in vitro* leaf callus extract has shown very potent activity against a broad range of bacteria and fungi.

CONCLUDING REMARKS

Hence, the study is designed to screen and identify the therapeutic suitability of this plant extract for the treatment of a particular disease. In the present study, potent activity has been reported in against twelve bacteria and eight fungi. The results of present study indicated that the callus extracts of *Canthium parviflorum* has potent antibiotic compounds which need to be isolated and characterized. This work is very useful to pharmaceutical industries for isolation and identification of new drugs with new mode of action.

ACKNOWLEDGEMENTS

The first author **Dr. Sirigiri Chandra Kala** is thankful to University Grants Commission, New Delhi for providing financial support with **Rajiv Gandhi National Fellowship**.

REFERENCES

1. Sirigiri Chandra Kala, Muvva Vijayalakshmi, Shaik Ibrahim Khalivulla and Kokkanti Mallikarjuna. (2014): Phytochemical and Antimicrobial Analysis of Callus Extracts of *Biophytum sensitivum* (Linn) DC. *British Microbiology Research Journal*, 4(8): 869-884.
2. Mohideen, S., Ilavarasan, R, Hemalatha, S. Anitha, N. and Sasikala, E. (2003): Wound healing and diuretic activities of *Canthium parviflorum* Lam. *Natural Product Sci*. 9(2), 102-104.
3. Parinitha Mahishi, B. H, Srinivasa and M. B. Shivanna (2005): Medicinal plant wealth of local communities in some villages in Shimoga District of Karnataka, India. *J. Ethnopharmacol*, 98(3): 307-312.

4. Anitha, B, Mohan, V. R, Athiperumalsami, T. and S. Sutha (2008): Ethnomedicinal plants used by the kanikkars of Tirunelveli District, Tamil Nadu, India to treat skin diseases. *Ethnobotanical Leaflet*, 12, 171-180.

5. Ayyanar, M, Sankarasivaraman, K. and Ignacimuthu, S. (2008): Traditional Herbal Medicines used for the treatment of Diabetes among two major tribal groups in south Tamil Nadu, India. *Ethnobotanical Leaflets*, 12: 276-280.

6. Sathish Kumar, T. Shanmugam, S. Palvannan, Bharathi Kumar, V. M. (2008): Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves, *Natural Product Radiance*, 7(2): 122-126.

7. Rajyalakshmi, P. (2003): *Canthium parviflorum*: an under exploited carotene rich fruit. *Natural product radiance*. 2(2): 70.

8. Haroled Peter, P. L, Abraham Shanjan, Godwin Blessing Issac, A, Sanjay Das and Arasan Elayaraja, 2011: Preliminary Phytochemical and Antimicrobial Screening of Whole Plant Extracts of *Canthium parviflorum* Lam. *Intern. Jour. Phytopharmacy Res. 2(1)*: 30-34.

9. Murashige T. and Skoog F (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol*. 150: 473-497.

10. Cappuccino, J. G. and Sherman, N. (2004): Microbiology, a laboratory manual. Pearson *Education, Inc,* New Delhi, pp, 282-283.

11. Volk, R. B. (2008): A newly developed assay for the quantitative determination of antimicrobial activity of both hydrophilic and lipophillic test compounds without any restriction. *Microbiol.* Res, 163: 161-167.

12. Arumugam Thangavel, Muniappan Ayyanar, Yesudason Justin Koil Pillai and Thangavel Sekar (2011): Phytochemical screening and antibacterial activity of leaf and callus extracts of *Centella asiatica. A Jour. the Bangladesh Pharmacol. Society* (BDPS), Bangladesh J. Pharmacol. 6: 55-60.

13. Faisal Hammad Mekky Koua, Hind Ahmed Babiker, Asim Halfawi, Rabie Osman Ibrahim, Fatima Misbah Abbas, Eisa Ibrahim Elgaali and Mutasim Mohamed Khlafallah Phytochemical (2011): Biological study of *Striga hermonthica* (Del.) Benth callus and intact Plant Res. in *Pharmaceutical Biotechnol*. Vol. 3(7), pp. 85-92, July, ISSN 2141-2324.

14. Alam, K, Parvez, N, Yadav, S, Molvi, K, Hwisa, N, S. M. Al. Sharif, Pathak, D. Murti, Y. and Zafar, R. (2011): Antimicrobial activity of leaf callus of *Bacopa Monnieri L. Scholars Res. Library Der. Pharmacia Lettre*, 3(1): 287-291.

15. Yamin Bibi, Muhammad Zia, Sobia Nisa and Darima Habib (2011): Abdul Waheed and Fayyaz M Chaudhary. Regeneration of *Centella asiatica* plants from non- embryogenic cell lines and evaluation of antibacterial and antifungal properties of regenerated calli and plants. *J. Biological Engg*, 5: 13.

16. Ravinder Singh, C. Antimicrobial effect of callus and Natural plant extracts of *Premna serratifolia* L. (2011): *Intern. J. Pharmaceut. and Biomed. Res.* (IJPBR), 2(1): 17-20.

17. Johnson, M, Babu, A. and Irudayaraj, V. (2011). Antibacterial studies on *in vitro* derived calli of *Ocimum basilicum* L. *J. Chem. Pharm. Res*, 3(1): 715-720.

18. Johnson, M, Maridass, M. and Irudayaraj V. (2008): Preliminary Phytochemical and Anti-Bacterial Studies on *Passiflora edulis*. *Ethanobotanical Leaflets*, 12: 425-432.

19. Viji, M. O. (2011): Antimicrobial and cytotoxic profile changes in leaf stem and Root Tissues of *Withania somnifera – Poshita* variety. Intern. *J. Pharmaceutical and Biomedical Res.* (IJPBR): 2(3): 81-89.

20. Chandra Kala, S, Mallikarjuna, K. and Aruna, P. (2012): Qualitative Phytochemical Analysis of Seed and Leaf Callus Extracts of *Canthium Parviflorum* Lam. Guntur District, Andhra Pradesh. *Int. J. Pharm. Bio. Sci*. 3(4): 177-182.

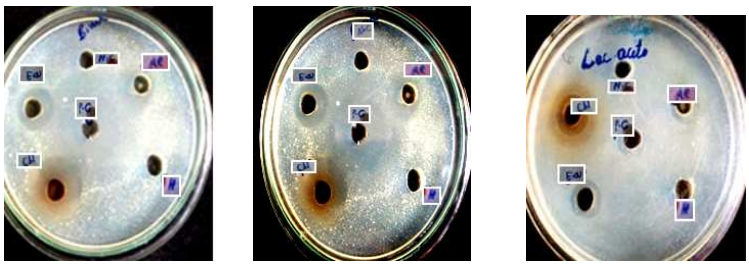
21. Amos, B. M. W, Tautua, I. S, Angaye, S. and Jonathan, G. (2012): Phytochemical Screening and Antimicrobial Activity of the Methanol and Chloroform Extracts of *Alchornea Cordifolia*. *J. Emerging Trends in Engg. and Applied Sciences (JETEAS)*, 2(3): 445-447.

22. Sathiya Priya, P, Sasikumar, J. M. and Gowsigan, G, 2009: Antibacterial activity of methanol extract of *Ruta chalapensis* (L), *Quercus infectoria* (Oliver) and *Canthium parviflorum* (Lam). *Ancient Sci. of Life*, 29**(**2): 28-31.

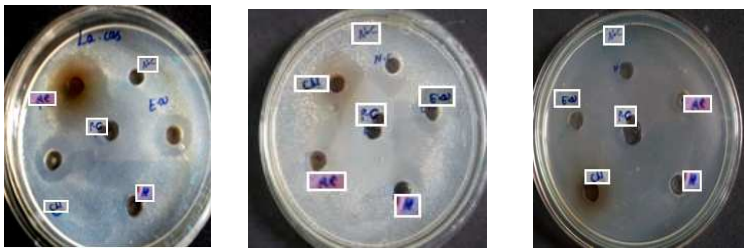
23. Jayasinghe, U. L. B, C. P. Jayasooriya, B. M. R. Bandara, S. P. Ekanayake, L. Merlini, G. Assante (2002): Antimicrobial activity of some Sri Lankan Rubiaceae and Meliaceae. *Fitoterapia* 73: 424-427.

APPENDICES

Figure 1: Antibacterial activity of leaf callus extracts of *Canthium parviflorum.*



A) *Bacillus megaterium* *B) Bacillus subtilis C) Lactobacillus acidophilus*

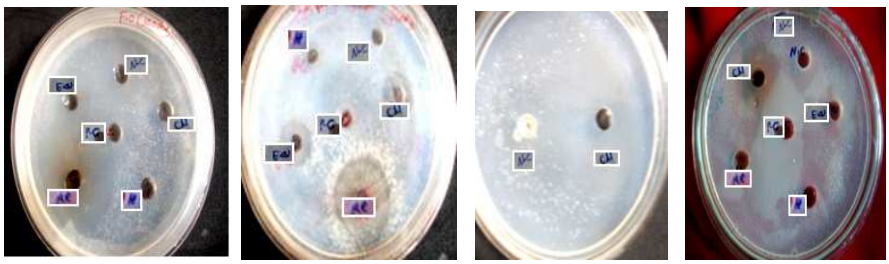


D) *Lactobacillus casei* E) *Staphylococus aureus* F) *Proteus vulgaris*

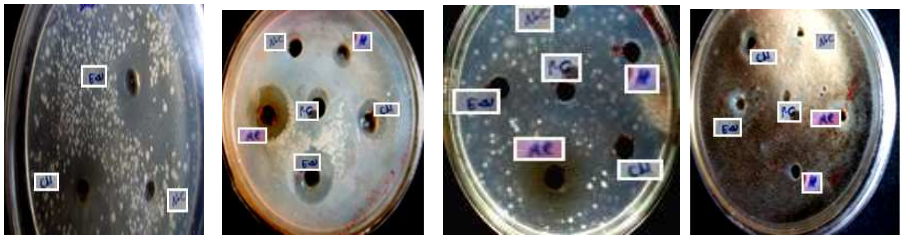
He: Hexane extract, **Chl:** Chloroform extract, **E.a:** Ethylacetate extract, **Me:** Methanol extract,

N.C: Negative control (Hexane, Chloroform, Ethylacetate, Methanol), **P.C:** Positive Control.

Figure 2: Antifungal Activity of Leaf Callus Extracts of *Canthium Parviflorum*



A) Fusarium oxysporum B) Rhizoctonia solani C)Trichoderma viride D) Candida albicans



E) Candida albicans F) Botrytis cinerea G) Helminthosporium solani H) Aspergillus niger

He: Hexane extract, **Chl:** Chloroform extract, **E.a:** Ethylacetate extract, **Me:** Methanol extract,

N.C: Negative control (Hexane, Chloroform, Ethylacetate, Methanol), **P.C:** Positive Control.

Table 1: Antibacterial Activity of Callus Extracts of *Canthium Parviflorum*

Name of Bacteria	Zone of Inhibition (mm)																				
	Hexane extract (ppm)				He (N.C)	Chloroform extract (ppm)				Cl (N.C)	Ethyl acetate extract (ppm)				E.a (N.C)	Methanol extract (ppm)				Me (N.C)	
	25	50	75	100	100	25	50	75	100	100	25	50	75	100	100	25	50	75	100	100	PC 0.1 ppm
B.m	3.24 ± 0.12	4.50 ± 0.15	9.62 ± 0.21	11.50 ± 0.37	-	8.22 ± 0.41	13.44 ± 0.58	15.20 ± 0.27	22.80 ± 0.30	0.40 ± 0.02	8.32 ± 0.38	10.45 ± 0.72	12.6 ± 0.12	18.52 ± 0.20	0.6 ± 0.20	8.6 ± 0.65	10.40 ± 0.34	13.68 ± 0.65	17.84 ± 0.24	0.8 ± 0.15	21.50 ± 0.20
B.s	-	-	-	3.32 ± 0.11	-	6.56 ± 0.26	7.11 ± 0.34	13.60 ± 0.74	16.92 ± 0.31	-	6.45 ± 0.74	14.52 ± 0.18	17.58 ± 0.65	20.42 ± 0.02	0.5 ± 0.02	10.80 ± 0.41	13.50 ± 0.12	13.22 ± 0.24	16.20 ± 0.16	0.6 ± 0.11	10.10 ± 0.50
P.a	5.26 ± 0.18	7.52 ± 0.63	12.20 ± 0.25	15.32 ± 0.16	-	8.58 ± 0.12	10.82 ± 0.16	11.55 ± 0.11	12.25 ± 0.18	-	6.10 ± 0.80	8.10 ± 0.36	10.26 ± 0.62	12.44 ± 0.15	0.6 ± 0.8	5.76 ± 0.18	8.95 ± 0.14	10.36 ± 0.14	12.54 ± 0.08	-	13.42 ± 0.80
S.a	5.36 ± 0.06	6.52 ± 0.46	8.32 ± 0.26	14.90 ± 0.08	-	10.30 ± 0.24	15.35 ± 0.46	19 .20 ± 0.31	20.56 ± 0.09	-	7.50 ± 0.25	10.32 ± 0.22	12.63 ± 0.46	15.24 ± 0.08	0.6 ± 0.8	6.62 ± 0.15	10.26 ± 0.64	13.80 ± 0.15	14.60 ± 0.24	-	12.22 ± 0.16
L.a	4.56 ± 0.32	5.42 ± 0.08	6.24 ± 0.11	13.26 ± 0.28	-	9.10 ± 0.22	10.35 ± 0.06	11.26 ± 0.42	14.56 ± 0.05	-	8.62 ± 0.08	10.28 ± 0.36	11.55 ± 0.06	12.25 ± 0.22	0.8 ± 0.8	8. 20 ± 0.18	8.26 ± 0.62	10.52 ± 0.12	12. 42 ± 0.10	-	7.36 ± 0.08
L.c	-	-	-	2.45 ± 0.12	-	2.68 ± 0.14	5.20 ± 0.62	8.35 ± 0.17	12.55± 0.26	-	3.22 ± 0.11	5.14 ± 0.10	8. 16 ± 0.35	12.26 ± 0.06	0.8 ± 0.5	8.52 ± 0.13	12.50 ± 0.18	18.22 ± 0.15	22.42 ± 0.05	-	10.30 ±0.30
E.c	-	-	4.35 ± 0.46	5.25 ± 0.46	-	2.66 ± 0.12	5.32 ± 0.11	13.8 ± 0. 24	15. 24 ± 0.33	-	2.60 ± 0.15	4. 65 ± 0.88	7.55 ± 0.32	10.22 ± 0.63	-	4.80 ± 0.25	8.52 ± 0.24	10.52 ± 0.46	18.84 ± 0.55	-	12.22± 0.04
S.m	-	-	-	-	-	7.55 ± 0.22	10.42 ± 0.12	11.25 ± 0.26	12.32 ± 0.11	-	10.28 ± 0.18	14.35 ± 0.15	20.62 ± 0.26	25.34 ± 0.17	0.6± 0.2	5.28 ± 0.11	10.25 ± 0.12	14.32 ± 0.16	16.50 ± 0.02`	-	11.30± 0.37
E.f	-	-	-	-	-	4.25 ± 0.15	10.26 ± 0.06	12.42 ± 0.22	18.28 ± 0.45	-	2.42 ± 0.16	5.20 ± 0.12	6.35 ± 0.18	8.10 ± 0.22	-	2.26 ± 0.52	4.62 ± 0.42	6.34 ± 0.08	14.52 ± 0.11	-	14.90 ±0.26
P.v	-	-	-	-	-	5.65 ± 0.08	13.55 ± 0.11	15.70 ± 0.17	20.55 ± 0.26	-	-	-	-	2.45 ± 0.06	-	-	-	4.52 ± 0.08	5.28 ± 0.04	-	7.88 ± 0.18
X.c	4.82 ± 0.11	5.66 ± 0.35	6.25 ± 0.22	7.32 ± 0.45	-	5.25 ± 0.42	7.35 ± 0.13	11.62 ± 0.10	19.28 ± 0.08	-	8.22 ± 0.11	12.45 ± 0.13	13.16 ± 0.08	14.90 ± 0.42	0.6 ± 0.8	6.24 ± 0.15	8.18 ± 0.17	12.44 ± 0.33	16.20 ± 0.10	0.4 ± 0.5	7.60 ±0.16
S.t	2.24 ± 0.11	3.45 ± 0.14	4.22 ± 0.16	5.30 ± 0.10	-	3.22 ± 0.24	12.42 ± 0.16	13.15 ± 0.08	14.68 ± 0.10	-	-	2.90 ± 0.11	3.22 ± 0.06	4.68 ± 0.14	-	-	3.20 ± 0.68	7.62 ± 0.08	11.45 ± 0.12	-	8.90±0.12

Each value represents the mean of triplicate analysis. Standard deviation was 0.5 for the values.

B.m- *Bacillus megaterium*, B.s- *Bacillus subtilis*, P.a- *Pseudomonas aeruginosa*, *S.a-Staphylococcus aureus*, *L.a-Lactobacillus acidophilus*, *L.c-Lactobacillus casei*, *E.c- Escherichia coli*, *S.m- Streptococcus mutans*, *E.f- Enterococcus faecalis*, *P.v- Proteus vulgaris*, *X.c- Xanthomonas campestris*, *S.t-Salmonella typhi*

Table 2: Antifungal Activity of Callus Extracts of *Canthium parviflorum*

Name of Fungi	Zone of Inhibition (mm)																				Positive Control Fluconazole Standard
	Hexane extract (ppm)				He (N.C)	Chloroform extract (ppm)				Cl (N.C)	Ethyl acetate extract (ppm)				E.a (N.C)	Methanol extract (ppm)				Me (N.C)	
	25	100	75	100	100	25	50	75	100	100	25	50	75	100	100	25	50	75	100	100	0.1 ppm
F.s	-	-	-	-	-	2.54 ± 0.12	5.86 ± 0.11	8.65 ± 0.06	12.25 ± 0.28	-	4.82 ± 0.14	8.92 ± 0.05	10.44 ± 0.15	12.52 ± 0.11	-	10.62 ± 0.02	18.52 ± 0.06	22.14 ± 0.10	29.54 ± 0.36	-	10.42 ± 0.18
F.o	4.33 ± 0.11	6.12 ± 0.28	8.55 ± 0.08	11.62 ± 0.17	-	5.45 ± 0.22	9.26 ± 0.06	15.24 ± 0.15	18.92 ± 0.26	-	6.33 ± 0.56	11.45 ± .08	9.68 ± 0.10	11.55 ± 0.12	-	4.68 ± 0.15	15.88 ± 0.17	22.82 ± 0.18	35.25 ± 0.22	-	12.82 ± 0.06
R.s	-	-	-	-	-	6.25 ± 0.10	9.58 ± 0.34	12.50 ± 0.18	16.68 ± 0.12	-	10.22 ± 0.08	13.56 ± 0.06	16.55 ± 0.42	19.78 ± 0.45	4.18 ± 0.12	17.22 ± 0.22	18.62 ± 0.17	24.65 ± 0.12	29.60 ± 0.17	0.52 ± 0.02	12.30 ± 0.39
T.v	2.55 ± 0.28	6.45 ± 0.36	9.22 ± 0.11	12.65 ± 0.33	-	12.18 ± 0.06	16.12 ± 0.22	20.10 ± 0.11	25.66 ± 0.08	-	4.25 ± 0.05	7.68 ± 0.11	10.42 ± 0.22	14.69 ± 0.18	-	10.16 ± 0.28	15.22 ± 0.12	19.25 ± 0.08	22.65 ± 0.16	-	16.60±0.68
H.s	-	-	-	2.50 ± 0.44	-	2.98 ± 0.06	5.76 ± 0.11	7.35 ± 0.28	10.28 ± 0.36	0.52 ± 0.12	6.62 ± 0.17	8.22 ± 0.25	10.12 ± 0.28	13.98 ± 0.42	0.46 ± 0.11	8.85 ± 0.13	15.20 ± 0.52	22.36 ± 0.14	30.15 ± 0.06	0.54 ± 0.11	12.32 ± 0.06
B.c	-	-	-	-	-	6.72 ± 0.15	9.86 ± 0.11	12.68 ± 0.86	15.65 ± 0.06	-	10.8 ± 0.82	13.62 ± 0.22	16.76 ± 0.62	19.55 ± 0.12	0.52 ± 0.33	17.82 ± 0.11	20.22 ± 0.83	24.63 ± 0.22	29.24 ± 0.28		12.88 ± 0.42
C.a	-	-	-	-	-	9.54 ± 0.14	11.82 ± 0.05	14.22 ± 0.12	17.55 ± 0.11	-	6.35 ± 0.15	8.22 ± 0.28	11.68 ± 0.16	14.65 ± 0.17	0.42 ± 0.14	-	-	-	-	-	14.4 ± 0.08
A.n	-	-	-	2.65 ± 0.11	-	-	-	-	-	-	3.66 ± 0.08	5.60 ± 0.22	6.84 ± 0.44	8.40 ± 0.11	-	2.85 ± 0.10	4.66 ± 0.05	6.28 ± 0.14	14.55 ± 0.34	-	10.50 ± 0.62

Each value represents the mean of triplicate analysis. Standard deviation was 0.5 for the values.

F.s-Fusarum solani, F.o-Fusarium oxysporum, R.s-Rhizoctonia solani, T.c- Trichoderma viride, H.s-Helminthosporium solani, B.c-Botrytis cinerea, C.a-Candida albicans, A.n- Aspergillus niger.